

**CLAIMS**

1. Capsules comprising an outer membrane of a divalent or trivalent metal ion salt of alginic acid, and an internal nucleus comprising a suspension of stem cells, genetically modified male and female somatic cells, ovarian follicular cells, gametes, ovarian follicles, mammalian embryos and/or a biocompatible and/or biodegradable polymer constituting an artificial extracellular matrix.
2. The capsules according to claim 1, wherein said cells are suspended in a gelatinous medium.
3. The capsules according to claims 1 or 2 characterised in that said mammalian stem cells, genetically modified male and female somatic cells, ovarian follicular cells, ovarian follicles, gametes, and mammalian embryos are auto-organised *in vitro* into three-dimensional parenchymatose, follicular or alveolar type structures, which allow the *in vitro* growth of tissues and multicellular structures functionally similar to organs present in the whole mammalian organism.
4. The capsules according to any of the claims 1 to 3, characterised in that said stem cells, genetically modified male and female somatic cells, ovarian follicular cells, gametes, ovarian follicles and

mammalian embryos are functionally similar to the organs found in the whole organism and able to synthesise and secrete hormones such as progesterone (P4) and 17 $\beta$ -oestradiol (E2) and other biologically active substances in quantities similar to that which said structures produce *in vivo*.

5. The capsules according to any of the claims 1 to 4 wherein said alginate membrane is gelatinous, bioerodible and is cross-linked on the inner and/or outer surfaces and/or on both surfaces.

6. The capsules according to any of the claims 1 to 5 characterised in that the alginate membrane may be cross-linked using cross-linking agents selected from: protamine sulphate or phosphate, poly-L-lysine bromohydrate, polyvinylamine, or chitosans.

7. The capsules according to any of the claims 1 to 6 wherein the outer membrane is constituted by divalent metal alginates selected from: calcium, barium, strontium, zinc or trivalent metals selected from: aluminium, iron, chromium.

8. The capsules according to any of the claims 1 to 7 characterised in that the alginate membrane contains a second or more cellular species.

9. The capsules according to claim 8 characterised in that the alginate membrane contains ovarian follicular

cells, one or more gametes, one or more ovarian follicles and one or more mammalian embryos.

10. The capsules according to claim 8 characterised in that the alginate membrane contains one or more female gametes also in various stages of development.

11. The capsules according to any of the claims 1 to 10 characterised in that said biocompatible and/or biodegradable polymer is a hydrophilic polymer selected from the group constituted by: glucans, scleroglucans, mannans, galactomannans, gellans, carrageenans, pectins, polyanhydrides, polyaminoacids, polyamines, xanthans, celluloses and derivatives thereof: carboxymethylcelluloses, ethylcelluloses, methylcelluloses, hydroxypropylcelluloses, hydroxypropylmethylcelluloses, polyvinylalcohols, carboxyvinylpolymers, starches, alpha, beta, gamma cyclodextrins and dextrin derivatives in general, collagens, chitins, chitosans, alginic acid, hyaluronic acid.

12. The capsules according to claim 11, characterised in that said polymers, in aqueous solution, are present in concentrations between 0.01% and 90% of the total capsule weight.

13. The capsules according to claim 12, characterised in that said polymers, in aqueous solution, are present

in concentrations between 0.5% and 50% of the total capsule weight.

14. The capsules according to claim 12, characterised in that the hydrophilic polymeric material, which constitutes the artificial extracellular matrix of said capsule nucleus, is xanthan gum having viscosity between 800 and 1200 cP.

15. The capsules according to any of the claims 1 to 14 characterised in that said capsules have diameters between 0.5 mm and 30 mm, with membrane thicknesses between 300  $\mu$ m and 5000  $\mu$ m.

16. The capsules according to claim 15 characterised in that said capsules preferably have diameters between 2 mm and 10 mm.

17. The capsules according to any of the claims 1 to 16 characterised in that said capsules weigh between 5 mg and 200 mg.

18. The capsules according to claim 17 characterised in that said capsules preferably weigh between 20 mg and 100 mg.

19. A kit for the preparation of capsules according to any of the claims 1 to 18 comprising disposable, sterile, non-sterile or sterilisable, preset and pre-packaged instrumentation, for single and or multiple preparations.

20. The kit according to claim 19 comprising salts of a divalent or trivalent ion, an alkaline metal alginate, in separate pre-measured packages.

21. The kit according to claim 20, further comprising a biocompatible and/or biodegradable hydrophilic polymer preferably selected from: glucans, scleroglucans, mannans, galactomannans, gellans, carrageenans, pectins, polyanhydrides, polyaminoacids, polyamines, xanthans, celluloses and derivatives thereof: carboxymethylcelluloses, methylcelluloses, hydroxypropylcelluloses hydroxypropylmethylcelluloses, polyvinylalcohols, carboxyvinylpolymers, starches, alpha, beta, gamma cyclodextrins and dextrin derivatives in general, collagens, chitins, chitosans, alginic acid, hyaluronic acid.

22. The kit according to claims 20 or 21, further comprising a cross-linking agent preferably selected from protamine sulphate or phosphate, poly-L-lysine bromohydrate, polyvinylamine, or chitosans.

23. The kit according to any of the claims 20 to 22, further comprising a culture medium preferably selected from: physiological solution (isotonic saline), glucosate solution, Basal Medium Eagle (BME) and derivatives thereof, Hanks salts solution and

derivatives thereof, tissue culture medium 199 (TCM 199) and derivatives thereof, phosphate buffered saline (PBS) and derivatives thereof, Krebs salts solution and derivatives thereof, Dulbecco modified Eagle's medium (DMEM) and derivatives thereof, tris-buffered medium (TBM) and derivatives thereof, Tyrode's salts solution and derivatives thereof, Modified sperm washing medium, modified human tubal fluid, Modified ham's F-10 medium, Upgraded B2 INRA medium, B2 INRA Menezo Medium, Upgraded B9 medium.

24. The kit according to any of the claims 20 to 23, further comprising extrusion devices such as sterile, non-sterile or sterilisable nozzles, needles or syringes.

25. The kit according to any of the claims 20 to 24, wherein said divalent or trivalent ion salts are selected from: salts of calcium, barium, strontium, zinc, aluminium, iron or chromium.

26. The kit according to any of the claims 20 to 25, wherein said alginate is sodium alginate.

27. A process for the preparation of capsules according to any of the claims 1 to 18 which comprises the following steps:

a) suspension of the cells in a culture medium or in an appropriate biological liquid, optionally containing

a biocompatible and/or biodegradable hydrophilic polymer;

b) the addition to the suspension thus obtained of a divalent or trivalent ion salt until the attainment of ion concentrations between 1 and 500 mmol/l;

c) extrusion of the cellular suspension through extruders, orifices, nozzles or needles having dimensions between 50  $\mu\text{m}$  and 5000  $\mu\text{m}$  into an alkaline metal alginate solution in culture medium, having a concentration between 0.01% and 5% w/v, kept stirring at a speed between 10 and 200 rpm;

d) optionally, cross-linking of the capsules thus formed, through interfacial polymerisation of the alginate using the cross-linking agents according to claim 5, at a temperature between 5° C and 40° C for a time between 1 minute and 120 minutes.

28. The process according to claim 27 further comprising the recovery stage of the capsules by filtration, the washing of the same and their suspension in culture medium.

29. The process according to claim 27 further comprising the preservation stage of the capsules under laboratory culture conditions, or by lyophilisation, refrigeration, freezing or cryopreservation.

30. The process according to any of the claims 27 to 29

further comprising the vehicularisation stage within said capsules of cells, tissues, tissue parts, organs, organ parts, cell cores, gametes and embryos either freshly removed or appropriately preserved.

31. The process according to claim 30 wherein said vehicularisation stage comprises the stage of injecting or microinjecting into said capsules, cells, tissues, tissue parts, organs, organ parts, cell cores, gametes and embryos at various stages of development, either freshly removed or appropriately preserved.

32. The process according to any of the claims 27 to 31 further comprising the incubation stage, in an appropriate culture medium, of said capsules with cell cores, tissues, organs or parts thereof, gametes and embryos.

33. The process according to any of the claims 27 to 32 further comprising the stage of aspiration or removal, with any means or any technique and during any developmental stage of the cell cores, tissues or organs or parts thereof, gametes, embryos or substances produced by them.

34. The process according to any of the claims 27 to 33 further comprising the stage of extraction, purification, characterisation and sequencing of the substances produced such as hormones, metabolites,

catabolites and other biologically active substances.

35. The process according to any of the claims 27 to 34 wherein, in step (a), said divalent or trivalent ion is calcium, barium, strontium, zinc, aluminium, iron or chromium chloride or sulphate at a concentration between 5 and 200 mmol/l.

36. The process according to any of the claims 27 to 35 wherein, in step (a), the culture medium used is selected from: physiological solution (isotonic saline), glucosate solution, Basal Medium Eagle (BME) and derivatives thereof, Hanks salts solution and derivatives thereof, tissue culture medium 199 (TCM 199) and derivatives thereof, phosphate buffered saline (PBS) and derivatives thereof, Krebs salts solution and derivatives thereof, Dulbecco modified Eagle's medium (DMEM) and derivatives thereof, tris-buffered medium (TBM) and derivatives thereof, Tyrode's salts solution and derivatives thereof, Modified sperm washing medium, modified human tubal fluid, Modified ham's F-10 medium, Upgraded B2 INRA medium, B2 INRA Menezo Medium, Upgraded B9 medium, optionally containing a biocompatible and/or biodegradable polymer which constitutes the artificial extracellular matrix.

37. The process according to any of the claims 27 to 36 wherein, in step (a), the medium used is TCM 199 and

derivatives thereof, containing a hydrophilic polymer constituting the artificial extracellular matrix.

38. The process according to any of the claims 27 to 37 wherein, in step (a), the cellular sediment dilution / polymeric solution volume ratio is between 1:0.05 and 1:200.

39. The process according to claim 38 wherein the cellular sediment dilution / polymeric solution volume ratio is between 1:0.1 to 1:100.

40. The process according to any of the claims 27 to 39 wherein, in step (c), the extruded cellular suspension and the alginate solution volume ratio is between 1:1 and 1:250.

41. The process according to any of the claims 27 to 40 wherein, in step (c), extrusion occurs through the use of automated, semi-automated microencapsulators, peristaltic or piston pumps or alternatives, or by the use of manually operated syringes at such a speed as to produce from 10 to 250 drops/minute.

42. The process according to any of the claims 27 to 40 wherein, in step (c), the extrusion, with automated, semi-automated microencapsulators, peristaltic or piston pumps or alternatives, or by the use of manually operated syringes, occurs at such a speed as to produce 60 drops/minute.

43. The process according to any of the claims 27 to 40 wherein, in step (c), the extrusion of the cellular suspension occurs through the use of needles with internal diameters between 300  $\mu\text{m}$  and 2000  $\mu\text{m}$ .

44. The process according to any of the claims 27 to 43 wherein, in step (c), the alginate solution is kept stirring at a speed between 20 and 100 rpm and has a concentration between 0.1% and 1% w/v.

45. The process according to any of the claims 27 to 44 wherein, in step (c), the extruded cellular suspension and the alginate solution volume ratio is between 1:15 and 1:50.

46. The process according to any of the claims 27 to 45 wherein said alginates, in a 2% solution in water and at a temperature of 25°C, have a viscosity between 200 cP and 20000 cP.

47. The process according to any of the claims 27 to 46 wherein steps (a), (b) and (c) are performed at a temperature between 5°C and 40°C.

48. The process according to any of the claims 27 to 47 wherein steps (a), (b) and (c) are performed at a temperature between 20°C and 30°C.

49. The process according to any of the claims 27 to 46 wherein step (d) is performed at a temperature between 5°C and 40°C, for times between 1 minute and 120

minutes.

50. The process according to any of the claims 27 to 46 wherein step (d) is performed at a temperature between 20°C and 30°C, for times between 3 minutes and 30 minutes.